PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/82, 5/04, 15/29, A01H 4/00, 5/00 | A1

(11) International Publication Number: **WO 99/40210**

(43) International Publication Date: 12 August 1999 (12.08.99)

(21) International Application Number: PCT/US99/02528

(22) International Filing Date: 5 February 1999 (05.02.99)

(30) Priority Data:
09/019,155 6 February 1998 (06.02.98) US

(71) Applicants: THE UNITED STATES OF AMERICA, as represented by THE SECRETARY OF AGRICULTURE [US/US]; 1400 Independence Avenue S.W., Washington, DC 20250–0302 (US). WASHINGTON STATE UNIVERSITY RESEARCH FOUNDATION [US/US]; N.E. 1615 Eastgate Boulevard, Pullman, WA 99163 (US).

(72) Inventors: SIMMONS, Mary, K., Walker; 111 Arrow, Moscow, ID 83843 (US). VERHEY, Steven, D.; 3216 French Loop N.W., Olympia, WA 98502 (US). HO, Tuan–Hua, D.; 1512 Baxter Lane Court, Chesterfield, MO 63017 (US). HOLAPPA, Lynn, D.; 1007 Harrison Street, Colton, WA 99113 (US).

(74) Agents: PENDORF, Stephan, A. et al.; Pendorf & Cutliff, P.O. Box 20445, Tampa, FL 33622–0445 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SUPPRESSION OF ALPHA–AMYLASE AND PROTEASE EXPRESSION USING A SERINE/THREONINE PROTEIN KINASE

(57) Abstract

The invention is directed to methods of altering the expresion of alpha–amylase and proteases without hormones, by altering the expression of protein kinases. In plants, suppression of alpha–amylase and proteases is achieved in the absence of abscisic acid by increasing the expression of protein kinases of the serine/threonine group, such as PKABA1, an abscisic acid (ABA)–responsive protein kinase. Increasing the expression of PKABA1 can be achieved by transforming the plant with a DNA sequence encoding at least one polypeptide having a PKABA1 protein kinase activity linked to a suitable promoter.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

SUPPRESSION OF ALPHA-AMYLASE AND PROTEASE EXPRESSION USING A SERINE/THREONINE PROTEIN KINASE

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to methods of altering the expression of alpha-amylase, proteases, and other hydrolytic enzymes. This invention relates to a method of restricting germination and maintaining dormancy in seeds by genetically engineering the seeds to contain a DNA sequence encoding at least one polypeptide having a serine/threonine protein kinase activity.

2. Description of Related Art

Plants must adapt to changing environmental conditions. Drought or extreme temperatures trigger large increases in the plant hormone abscisic acid (ABA) and subsequently induce ABA-responsive genes. ABA has been implicated in the control of a wide range of essential physiological processes including seed development and plant adaptation to environmental stress. Plant mutants lacking the ability to produce or respond to ABA are far less capable of surviving environmental stress. The steps in the transduction pathway from ABA increases to stress-responsive gene expression are not known. One possible link in this signal transduction pathway is an ABA-regulated protein kinase. In animals and yeast, protein kinase activity often mediates a cellular adjustment to external stimuli, and a similar activity in plants is likely.

While many ABA-inducible genes have now been cloned, the function of these genes in adaptation to environmental changes is not yet clear. A number of ABA-responsive genes encode late embryogenesis abundant (LEA) proteins that accumulate in drying seeds and in water-stressed seedlings. In wheat and barley the

LEA proteins include EM, dehydrin (RAB), HVA1 group 3 LEA, and group 3 LEA (II) (Curry and Walker-Simmons, 1993, In: Plant Responses to Environmental Stress, eds. T.V. Close and E.A. Bray, pp 128-136). Many of the LEA proteins are hydrophilic and may bind water and ions tightly to function as protectants against desiccation . The maize LEA protein RAB-17 has been found to be highly phosphorylated in vivo. In vitro experiments show that RAB-17 can be phosphorylated by a maize casein kinase II at a serine-cluster region of the protein. Whether phosphorylation affects the cellular function of RAB-17 or other LEA proteins has not been determined (Goday, et al., 1994, Plant Cell 6:351).

Plant responses to changing environmental, developmental and metabolic conditions can be mediated by protein kinases and reversible phosphorylation. During these responses protein kinase activities are often regulated by post-translational modifications such as protein phosphorylation that result in activation/deactivation of enzyme activities. Changes in protein kinases mRNA levels have not been often reported, but there is increasing evidence that some plant protein kinases involved in responses to external signals can be regulated at the transcript level (Anderberg and Walker-Simmons, 1992, Proc. Natl. Acad. Sci., 89:10183; Holappa and Walker-Simmons, 1997, Plant Mol. Biol. 33(5):935).

In germinating seeds, the plant hormone, gibberellin (GA), stimulates expression of the major starch digesting enzyme, alpha-amylase. Similarly, GA stimulates other hydrolytic enzymes including those that break down proteins (proteases). Action of these hydrolytic enzymes enables seeds to germinate and grow. Regulation of these enzymes is critical for controlling seed germination, malting quality in barley and preventing sprouting damage in wheat. The plant hormone, abscisic acid (ABA) has been demonstrated to have an important role in regulating seed

embryonic maturation and germination. Exogenously applied ABA prevents embryonic germination, blocks the expression of germination-specific enzymes, and promotes embryonic development. ABA is an effective germination inhibitor and can suppress expression of alpha-amylase and other hydrolases.

Involvement of ABA in regulating embryonic germination and the activity of germination enzymes is of particular importance in wheat. Germination of wheat before harvest can severely reduce the quality of the grain for food products resulting in large economic loss. This preharvest sprouting damage is caused by hydrolysis of starch by alpha-amylase accompanied by proteolytic damage of grain proteins. Both alpha-amylase activity and synthesis in cereals have been shown to be regulated by ABA through inhibition of gibberellic acid-induction of alpha-amylase synthesis (Nolan et al., 1987 Plant Mol. Biol. 8:12; Skriver et al. 1991 Proc. Natl. Acad. Sci. 88:7266). Malting of cereal grains produces enzymes that hydrolyze starch reserves of the seed endosperm to make sugars available for fermentation. Rate of production of alpha-amylase is important along with proteases and other hydrolytic enzymes. Seed dormancy and viability levels have critical effects on malting quality (Beckrich and Denault, 1987, "Enzymes in the preparation of beer and fuel alcohol" in Enzymes and their Role in Cereal Technology, ed. J.E. Kruger, D. Lineback, C. Stauffer, 1987, AACC, 335-355; Fincher and Stone, 1993, In: Barley Chemistry and Technology, eds. A.W. MacGregor and R.S. Bhatty, pp 247-295).

Sprouted wheat produces a poorer quality of flour compared with that obtained from unsprouted wheat. When the poorer quality flour is used in the baking of bread, the bread has a certain stickiness which causes trouble when slicing machines are used to slice the bread. Sprouting of wheat is associated with an increase in the amount of alpha-amylase, a starch degrading

enzyme. Since the increased level of alpha-amylase is a major factor associated with wheat sprouting, it has been implied by different authors that it should be possible to improve baking properties of sprout-damaged wheat flour by reducing alpha-amylase activity. A number of different physico-chemical and chemical factors have been studied as potential alpha-amylase inhibitors or inactivators (Zawistowska, US Patent No. 5,047,257). A number of compounds were found to suppress alpha-amylase effects, but some also affected sensitive gluten proteins by changing their structure and functional properties which are important for baking performance and caused deterioration of other technological characteristics of bread. Moreover, the application of different chemicals such as alpha-amylase inhibitors or inactivators as food additives is limited due to the toxicity of some of them. A method of reducing alpha-amylase activity in the seed would eliminate the need for additives in the flour.

Protein kinases are critical components in signal transduction pathways leading to cellular adjustments in response to changes in extracellular conditions. Protein kinases and their phosphorylated intermediates modulate many plant physiological processes, including the perception of external signals such as phytohormones, environmental changes, and light. For example, protein kinases have roles in phytohormone responses as indicated by ethylene-mediated responses. Arabidopsis mutants that fail to respond to ethylene or that constitutively display the ethylene response have been found to have protein kinase gene mutations, and ethylene responses in wild-type Arabidopsis are blocked by protein kinase inhibitors. Protein kinases also have roles in responses to changing environmental conditions. For example, osmotic stress results in the phosphorylation of unique sites on sucrose phosphate synthase in spinach leaves, and low temperature and salt stress result in the accumulation of phosphorylated

proteins. The plant hormone ABA is a signal common to the initiation of many environmental stress responses and there is increasing evidence that protein kinases have a role in ABA-mediated responses. ABA affects protein phosphorylation of Lemna chromatin proteins and carrot embryonic proteins. Additionally, an Arabidopsis mutant with a defective protein phosphatase 2C is insensitive to ABA and has reduced ABA-mediated responses (Goday et al. 1994, Plant Cell 6:351).

PKABA1 from wheat was one of the first plant protein kinase genes to be identified that is regulated at the mRNA level in response to environmental stress and the plant hormone ABA (Anderberg and Walker-Simons, 1992, Proc. Natl. Acad. Sci. 89:10183). PKABA1 mRNA accumulates in wheat seedling tissue subjected to dehydration, cold temperatures, osmotic stress and high salt and may be part of a general response mechanism to environmental stress (Holappa and Walker-Simmons, 1995, Plant Physiol. 108:1203).

PKABA1 kinase has the conserved features of a serine-threonine protein kinase with a unique feature of a stretch of acidic (aspartic) amino acid residues beyond the catalytic domain near the carboxyl terminus. The accumulation of PKABA1 mRNA may be part of the initial stress response to environmental signals mediated by ABA that ultimately result in the accumulation of osmoprotectants (Holappa and Walker-Simmons, 1995, Plant Physiol. 108:1203).

SUMMARY OF THE INVENTION

The invention is directed to altering the expression of alpha-amylase and proteases without hormones, by altering the expression of protein kinases. In plants, suppression of alpha-amylase and proteases is achieved in the absence of abscisic acid by increasing the expression of protein kinases of the

serine/threonine group, such as PKABA1, an abscisic acid (ABA)-responsive protein kinase. Increasing the expression of PKABA1 can be achieved by transforming the plant with a DNA sequence encoding at least one polypeptide having a PKABA1 protein kinase activity linked to a suitable promoter. Alternatively, an increase in alpha-amylase expression could be achieved by reducing the expression of PKABA1. Reducing PKABA1 expression may be achieved by transforming the plant with an antisense PKABA1 construct or by transforming the plant with the sense PKABA1 and selecting for transformants having reduced PKABA1 activity due to gene splicing.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing that PKABA1 suppresses gibberellin (GA)-stimulated EPB-1 protease gene expression.

FIG. 2 is a graph showing the effect of increasing the ratio of the PKABA1 construct to the gibberellic acid response complex of alpha-amylase (GARC-GUS) construct (MBL022) on GA-induced alpha-amylase expression. MBL022 consists of the truncated promoter of a low pI alpha-amylase gene, Amy32b.

FIGS. 3A and 3B are graphs showing that PKABA1 induces ABA-responsive gene expression in the absence of the ABA phytohormone.

FIG. 4 shows that the calcium-dependent protein kinase CDPKci does not suppress alpha-amylase gene expression.

FIG. 5 shows that the calcium-dependent protein kinase CDPKna does not suppress alpha-amylase gene expression.

DEFINITIONS

The term "plant" includes whole plants, plant organs (e.g., leaves, stems, roots, flowers, etc.), seeds and plant cells and progeny of same. The class of plants which can be used in the methods of the invention is generally as broad as the class of

higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants.

The term "genetically engineered DNA sequence" refers to a DNA sequence which has been manipulated by genetic engineering methods such as recombinant DNA techniques known to those of skill in the art.

The term "polypeptide having a serine/threonine protein kinase activity" refers to any polypeptide which is capable of using gamma-phosphate of adenosine triphosphate (ATP) or guanosine triphosphate (GTP) to generate phosphate monoesters utilizing protein alcohol groups (on serine and threonine) as phosphate group acceptors. These enzymes all have a similar kinase domain (catalytic domain) that consists of 200-300 amino acid residues. Functions of the kinase domain include binding of the ATP (GTP) phosphate donor, binding of a protein (or peptide) substrate and transfer of the gamma-phosphate from ATP (GTP) to the acceptor hydroxyl of the serine or threonine residue of the protein substrate.

The term "polypeptide having a CaMK group protein kinase activity" refers to any polypeptide which is capable of phosphorylating serine/threonine residues near basic amino acid residues, particularly arginine and lysine. These include serine/threonine protein kinases with sequence similarity to calmodulin-dependent protein kinases.

The term "polypeptide having a PKABA1 protein kinase activity" refers to any polypeptide which is capable of suppressing alpha-amylase and protease expression and which has sequence similarity to PKABA1.

DETAILED DESCRIPTION OF THE INVENTION

During the germination of cereal grains, the embryo synthesizes and secretes gibberellins (GA) to the aleurone layer

where GA induces the expression of alpha-amylase and protease genes. Expression of these genes is suppressed by abscisic acid (ABA) synthesized during seed development or under unfavorable conditions. However, ABA also induces the expression of genes encoding late embryogenesis abundant (LEA) proteins. PKABA1, a member of a unique serine/threonine sub-family of protein kinases, exhibits up-regulation in response to ABA and environmental stresses. The involvement of PKABA1 in the signal transduction pathways mediating ABA induction and suppression of gene expression has been studied using the barley aleurone system.

Previous attempts to affect alpha-amylase production in cereals have used small, non-proteinaceous compounds such as plant hormones. Difficulties in controlling hormones have made such attempts inconsistent. The instant invention is novel and unexpected in that it utilizes a protein kinase in the absence of hormones, and involves transformation instead of exogenous addition of hormones. Serine/threonine protein kinases have been shown to inhibit gibberellin induced expression of hydrolytic enzymes including alpha-amylase and EPB-1, a protease (Koehler and Ho, 1990, Plant Cell 2:769). In one embodiment, the protein kinase is a serine/threonine protein kinase. In a further embodiment, the protein kinase is from the CaMK group. In a preferred embodiment, the protein kinase is PKABA1 from wheat. In another embodiment, the protein kinase is the rat calmodulin dependent protein kinase.

The deduced amino acid sequence of PKABA1 has extensive homology to all 12 conserved catalytic subdomains characteristic of serine/threonine protein kinases but has a unique stretch of aspartic acid residues near the carboxyl terminus. The up-regulation of PKABA1 at the transcriptional level is unusual because protein kinases are usually regulated at the post-translational level. The PKABA1 sequence has similarity with

several protein kinases from animals, yeast and plants. These protein kinases include soybean calcium-dependent protein kinase, yeast SNF1 protein kinase, rat calcium/calmodulin-dependent protein kinase type II, β subunit, and yeast nim1$^+$ protein kinase (Anderberg and Walker-Simmons, 1992, Proc. Natl. Acad. Sci. 89:10183).

Serine/threonine plant protein kinases fall into three distinct groups with respect to the PKABA1 sequence. These three groups are the PKABA1 subfamily containing closely related kinase sequences with the characteristic acidic amino acid sequence at the carboxyl terminal end, the SNF1 (Suc nonfermenting) kinase subfamily, and the other plant Ser/Thr protein kinases. Some of these kinases with similar sequences to PKABA1 also appear to be involved in responses to changes in environmental or nutrient conditions and to exhibit a degree of transcriptional regulation.

Two groups of constructs were introduced to barley aleurone layers using particle bombardment: the reporter constructs, containing the coding sequence of β-glucuronidase gene driven by promoters of ABA- or GA-inducible genes, and the effector constructs, containing the coding region of protein kinases driven by a constitutive promoter. Over-expression of PKABA1 drastically suppressed the GA induction of alpha-amylase and protease genes and had a small effect on ABA induction of LEA genes. It was unexpected that PKABA1 alone, in the absence of hormones such as ABA, would suppress alpha-amylase and proteases.

When a PKABA1 construct was bombarded alone into barley aleurone layers and the seeds incubated with GA, the PKABA1 suppressed the expression of a protease, EPB-1, as shown in FIG. 1. Bombardment with EPB-1 alone, followed by GA treatment caused a 12x increase in the endopeptidase gene expression as measured by GUS activity. Co-bombardment of PKABA1 with EPB-1 reduced the GA-mediated stimulation to a 4-fold increase.

FIG. 2 shows the effect of increasing the ratio of PKABA1 construct to the tester construct (gibberellic acid response complex (GARC)-GUS of alpha-amylase) on alpha-amylase gene expression (GUS activity). The tester construct, MBL022 has been previously described (Lanahan et al. 1992, Plant Cell 4:203). Higher amounts of the PKABA1 construct completely suppress alpha-amylase gene expression. Similar effects were found with promoters for both low and high pI alpha-amylases.

FIGS. 3A and 3B show that PKABA1 induces ABA-responsive gene expression in the absence of the ABA phytohormone. The Qs264 construct contains the HVA1 gene which is a putative osmoprotectant in plants and is ABA-responsive. FIG. 3A shows that co-bombardment of the PKABA1 construct with the Qs264 construct causes a 4-fold increase in expression of HVA1 (as measured by GUS activity) in the absence of ABA. FIG. 3B shows that while co-bombardment with PKABA1 and Qs264 causes an increase in ABA-responsive gene expression, it is not as effective as ABA.

FIG. 4 and FIG. 5 show that bombardment with two other plant protein kinases was not effective in suppression of alpha-amylase expression. CDPKci and CDPKna are constructs containing different calcium-dependent protein kinases.

These results suggest that PKABA1 acts as an intermediate in the ABA suppression of GA-inducible gene expression in cereal aleurone layers.

The construct containing DNA encoding at least one polypeptide having a PKABA1 activity was used for wheat transformation according to established bombardment protocols (Weeks, et al., 1993, Plant Physiol. 102:1077). It is expected that the transgenic wheat plants will express reduced levels of alpha-amylase in the seeds. The seeds will be germination inhibited and remain dormant. In order to induce the seeds to germinate, high levels of germination-inducing hormones such as

GA, or protein kinase inhibitors will be added. Such transgenic plants and seeds would be useful for preventing sprouting damage in cereals, and to induce germination only when desired.

Transformation with an antisense PKABA1 construct would be useful for reducing the levels of PKABA1 in mature seed embryos and aleurone, which would potentially increase the rate of alpha-amylase activation in hydrated grain. This could achieve a seed with increased germination rates and more rapid increases in alpha-amylase and protease activity. These strategies can be used to enhance grain malting quality and increase seed viability and vigor.

Reducing PKABA1 may also be achieved by gene silencing. Plants could be transformed with sense constructs of PKABA1 and then those with reduced PKABA1 levels would be selected.

Another protein kinase, the rat calmodulin dependent protein kinase (CaMPK) has been shown to suppress alpha-amylase in barley aleurone.

EXAMPLE

A. Construction of the PKABA1 plasmid.

The construct was prepared using DNA from three sources, pKABA1, TaPK3 and pAHC17. The main source of insert DNA came from PKABA1 (GenBank Accession No. M94276). PKABA1 is a partial cDNA (Anderberg and Walker-Simmons, PNAS-USA 89:10183, 1992). We deduced the missing 5' information (comprising 10 amino acids) from a genomic clone for a closely related kinase, TaPK3 (Holappa and Walker-Simmons, Plant Molecular Biology 33:935, 1997) (GenBank Accession No. U29094). Using this information we made a double-stranded oligonucleotide "patch" for the 5' end of PKABA1, yielding a reconstituted chimeric PKABA1 cDNA. This reconstituted PKABA1 sequence was then cloned into the vector pAHC17, which

contains the ubiquitin promoter and first exon from the maize ubiquitin gene (Christensen and Quail, Transgenic Research 5:213, 1996).

B. Transformation of barley aleurone with the PKABA1-containing plasmid.

The transient gene expression method of Shen and Ho (Plant Cell, 1995, 7:295-307) was used. The reporter constructs contained the coding sequence of the beta-glucuronidase (GUS) gene driven by promoters of the GA-inducible Amy32b (alpha-amylase gene), GA- inducible EPB-1 (cysteine proteinase) and ABA-inducible HVA1 (LEA) gene. The effector construct was comprised of the coding sequence of PKABA1 linked to the 5'end of TaPK3 driven by a constitutive promoter (ubiquitin) described above. The reporter and effector gene constructs were inserted into barley aleurone layers by particle bombardment. The bombarded aleurone layers were incubated in hormone (ABA or GA) or control solutions for 24 hours. Then, the bombarded aleurone layers were homogenized and assayed for GUS activity with a fluorometer.

We claim:

1. A method for restricting germination and maintaining dormancy in seeds comprising:
   a. preparing a genetically engineered DNA sequence encoding at least one polypeptide having a serine/threonine protein kinase activity;
   b. operably linking said DNA sequence to a promoter for said DNA;
   c. transforming a transformable, regenerable plant cell to contain said operably linked DNA sequence of step b.

2. The method of claim 1 wherein said plant cell is from a monocot.

3. The method of claim 2 wherein said monocot is selected from the group consisting of wheat, barley, maize, rice and Bromus species.

4. The method of claim 1 wherein said genetically engineered DNA sequence encodes at least one polypeptide having a CaMK group protein kinase activity.

5. The method of claim 4 wherein said plant cell is from a monocot.

6. The method of claim 5 wherein said monocot is selected from the group consisting of wheat, barley, maize, rice and Bromus species.

7. The method of claim 1 wherein said genetically engineered DNA sequence encodes at least one polypeptide having a PKABA1 protein kinase activity.

8. A method for reducing gibberellin induction of hydrolytic enzymes and/or proteases and promoting the expression of abscisic acid-induced genes comprising:
   a. preparing a genetically engineered DNA sequence encoding at least one polypeptide having a serine/threonine protein kinase activity;
   b. operably linking said DNA sequence to a promoter for said DNA;
   c. transforming a transformable, regenerable plant cell to contain said operably linked DNA sequence of step b.

9. A transgenic plant exhibiting suppressed alpha-amylase gene expression comprising a genetically engineered DNA sequence encoding at least one polypeptide having a serine/threonine protein kinase activity, wherein said polypeptide suppresses gibberellin induction of alpha-amylase.

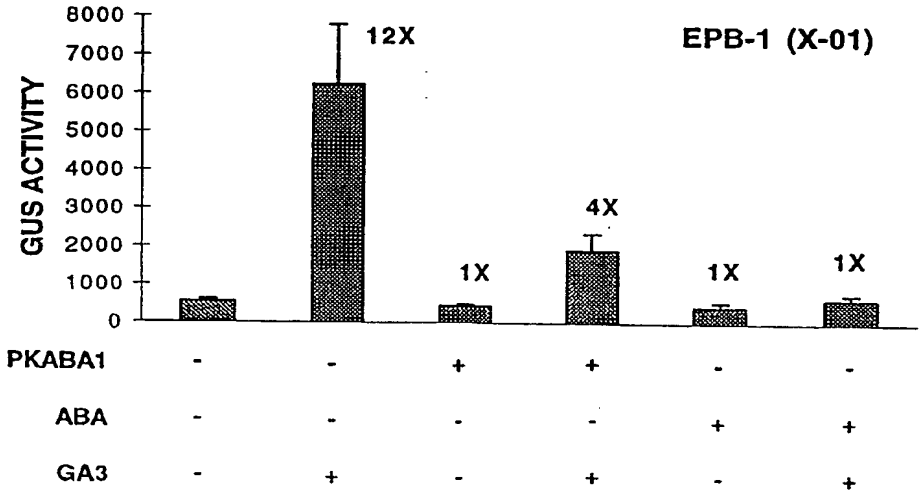
10. Seed from the transgenic plant of claim 9.

11. The transgenic plant of claim 9 wherein said genetically engineered DNA sequence encodes at least one polypeptide having a CaMK group protein kinase activity.

12. The transgenic plant of claim 9 wherein said genetically engineered DNA sequence encodes at least one polypeptide having a PKABA1 protein kinase activity.

13. A transgenic plant exhibiting suppressed alpha-amylase gene expression comprising a genetically engineered DNA sequence encoding at least one polypeptide having a PKABA1 protein kinase activity, wherein said polypeptide suppresses gibberellin induction of alpha-amylase.

14. Seed from the transgenic plant of claim 13.



EFFECT OF PKABA1 AND ABA ON THE EPB-1 RESPONSE TO GA3 TREATMENT

FIG. 1

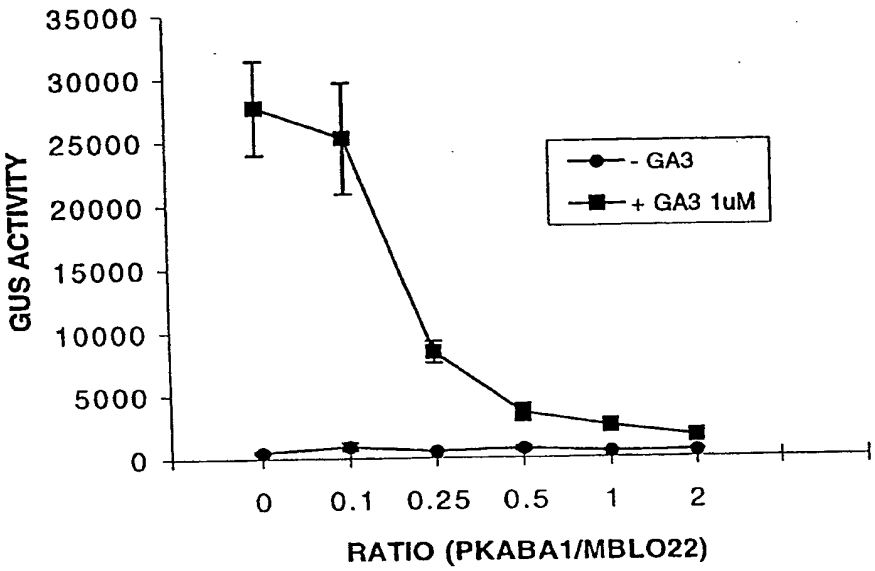

35000
30000
25000
20000
15000
10000
5000
0
GUS ACTIVITY
- GA3
+ GA3 1uM
0
0.1
0.25
0.5
1
2
RATIO (PKABA1/MBLO22)

FIG. 2

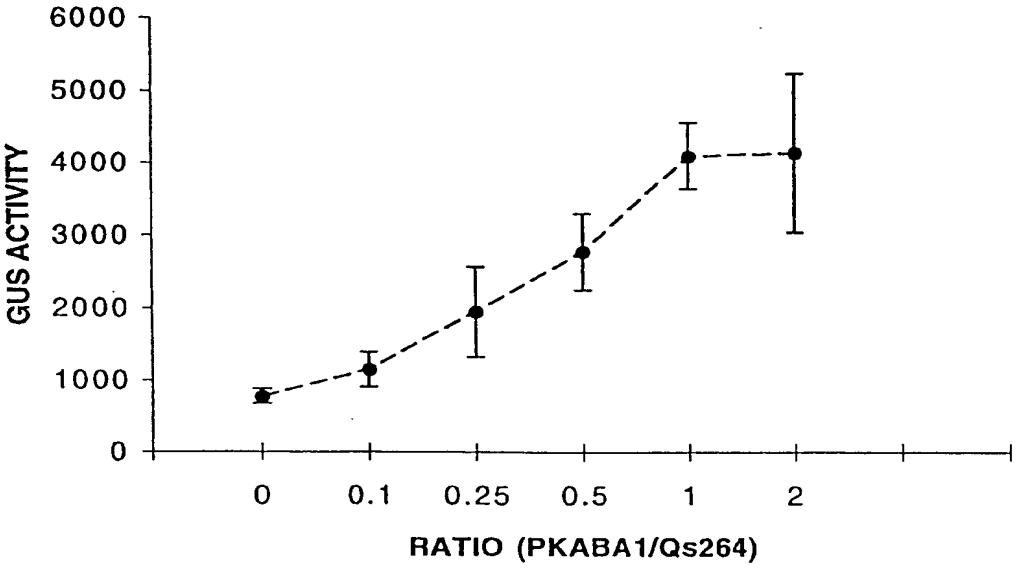

6000
5000
4000
3000
2000
1000
0
GUS ACTIVITY
0
0.1
0.25
0.5
1
2
RATIO (PKABA1/Qs264)

FIG. 3A

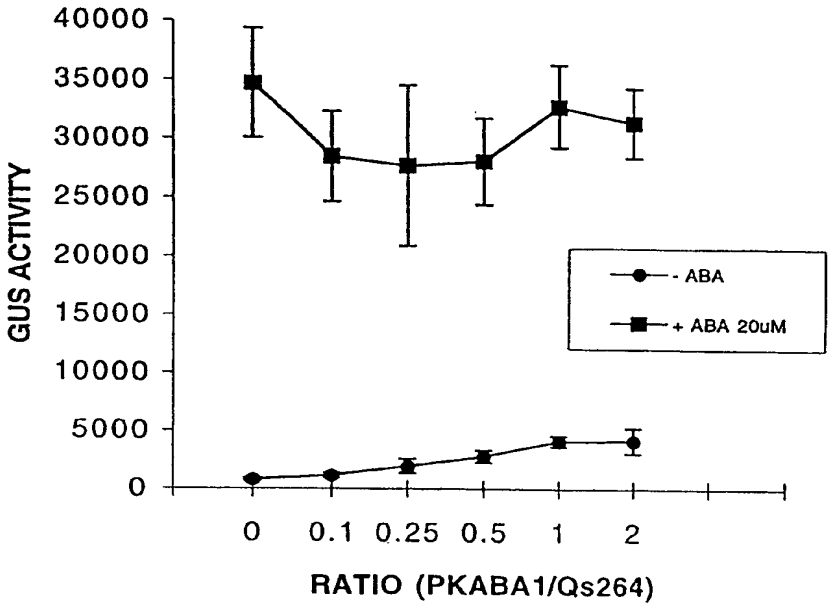

40000
35000
30000
25000
20000
15000
10000
5000
0
GUS ACTIVITY
- ABA
+ ABA 20uM
0
0.1
0.25
0.5
1
2
RATIO (PKABA1/Qs264)

FIG. 3B

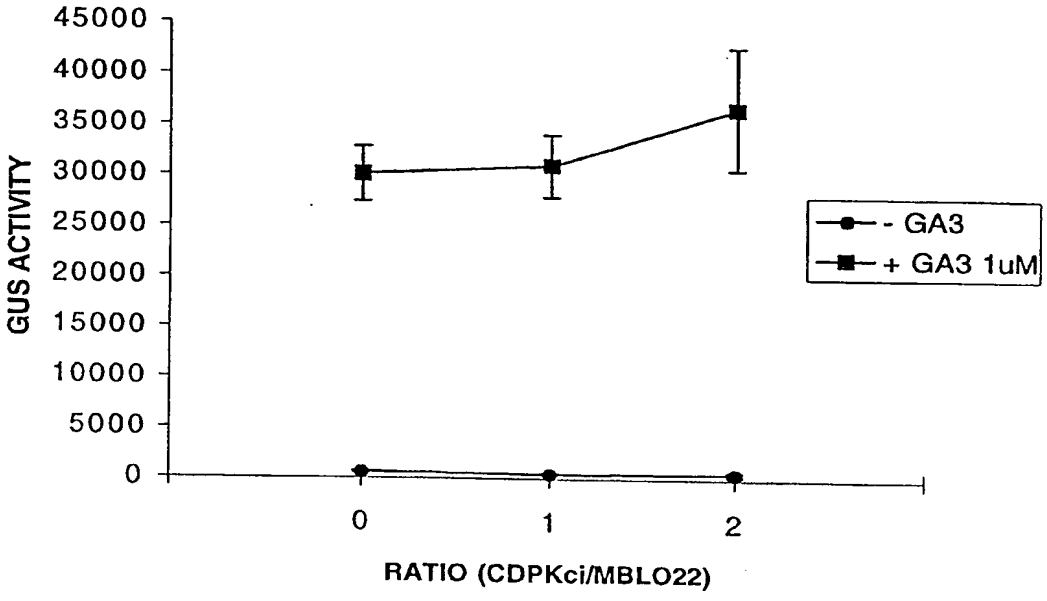

45000
40000
35000
30000
25000
20000
15000
10000
5000
0
GUS ACTIVITY
0
1
2
RATIO (CDPKci/MBLO22)
- GA3
+ GA3 1uM

FIG. 4

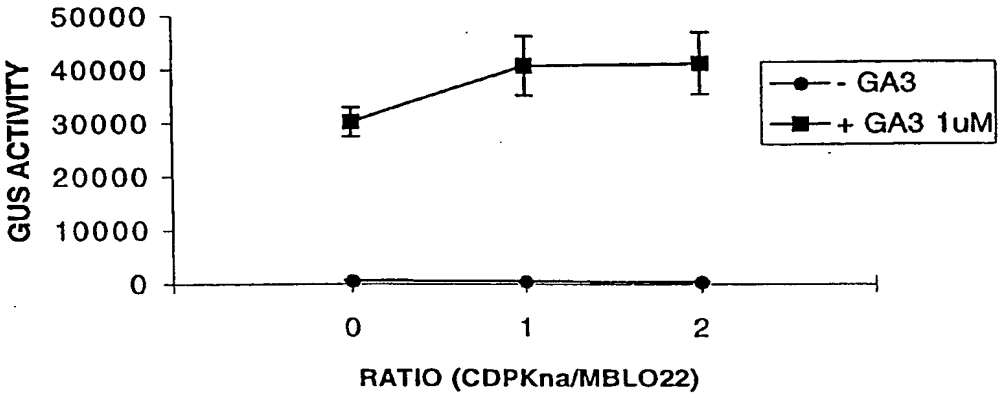

50000
40000
30000
20000
10000
0
GUS ACTIVITY
0
1
2
RATIO (CDPKna/MBLO22)
- GA3
+ GA3 1uM

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/02528

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/82, 5/04, 15/29; A01H 4/00, 5/00

US CL :435/69.1, 468, 410, 411, 419; 800/278, 284, 320, 320.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 468, 410, 411, 419; 800/278, 284, 320, 320.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

AGRICOLA, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANDERBERG et al. Isolation of a Wheat cDNA Clone for an Abscisic Acid-Inducible Transcript with Homology to Protein Kinases. Proc. Natl. Acad. Sci. USA. November 1992, Vol. 89, pages 10183-10187, see entire document.	1-14
Y	CHENG et al. Genetic Transformation of Wheat Mediated by Agrobacterium Tumefaciens. Plant Physiol. 1997, Vol. 115, pages 971-980, see entire document.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 MARCH 1999

Date of mailing of the international search report

31 MAR 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

OUSAMA M-FAIZ ZAGHMOUT

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*